## 1.0 Introduction

1.1 Background

Over the past decade, the investigation of the cause of human cancer has identified two categories of genetic events leading to cancer: the loss by mutation of a tumor suppressor gene and the activation of a tumor promoter gene. The most commonly mutated tumor suppressor gene is the p53 gene, found in approximately fifty percent of tumors.[1] This gene, located on the short arm of human chromosome 17, encodes a 53-kD phospho-protein involved in regulation of the cell cycle, and thus cell division. In the event of DNA damage, wild type p53 either induces a $G_1$ arrest to allow the cell to repair itself, or initiates apoptosis (programmed cell death). Conversely, mutations in the p53 gene have been associated with resistance to apoptosis and carcinogenesis. Aberrant forms of p53 protein are correlated with more aggressive tumors, metastasis, and lower survival rates. This is the case for cancers of the colon, esophagus, breast, lung, cervix, skin, parotid, bladder, and prostate.[6,7,8,9,10] Clinical studies have indicated that p53 status has prognostic value.[2,3,4,5]

In malignant cells, p53 mutations occur throughout the gene. They are mostly missense mutations leading to amino-acid substitutions in the protein, which usually result in synthesis of a mutant protein with increased stability but impaired function. This can easily be measured using immunological techniques. In a minority of cases, frameshift or chain-terminating (nonsense) mutations result in transcription of truncated or unstable p53 protein, which cannot usually be detected by immunological assays.

Adenovirus Type 5 is a mild pathogen in humans, most often causing minor respiratory symptoms. Ninety to 100% of adults are sero positive to adenovirus Type 5 indicating childhood infection and lifelong immunity. Adenovirus also causes cystitis in severely immuno-compromised patients, usually in the setting of allogeneic bone marrow transplantation.

An adenoviral vector with the necessary expression cassette containing human wild-type p53 gene has been constructed (Ad5CMV-p53). Pre-clinical studies showed that various cancer cell lines could be transduced with Ad5CMV-p53 (INGN 201). Injection of the wild-type p53 gene into human tumor cells with a mutant p53 genotype *in vitro* and *in vivo* has confirmed expression and function of the transgenic product in addition to correlation with tumor regression and survival improvement in animal models.[11,12,13]

Preclinical and early Phase I/II studies of intratumoral injections of INGN 201 also reveal systemic distribution of the adenoviral vector and subsequent rise in anti-adenoviral antibody titers. Antibody levels after multiple intratumoral injections were compared to baseline levels and were found to have risen up to 1000 fold without abrogating gene transfer or clinical anti-tumor activity. Biologically active INGN 201 was recovered from plasma after repeated doses and subsequent to rising antibody development, indicating the antibodies were not neutralizing. INGN 201 has been safely administered for up to six months despite the presence of antibodies.

The safety of intratumoral administration of INGN 201 has been well documented. The safety of an intravenous (i.v.) usage in humans has not been studied. An i.v. study in rats was conducted to assess systemic toxicity and absolute biodistribution. This study found elevated liver enzymes and histopathologic changes in the liver at higher doses. The biodistribution was generalized, with higher concentration in the liver. No significant toxicity was noted in other organ systems despite evidence of widespread exposure, and in some cases, persistence of vector for a duration of at least 15 days after administration.[14] The extrapolation of the animal data for risk assessment related to adenoviral dissemination in humans is uncertain. The data do provide solid preclinical evidence in support of an acceptable safety profile of INGN 201 administered i.v.. Phase I studies impart additional support for the safety of INGN 201, and a strong rationale for an intravenous study in view of the persistence, in plasma, of biologically active vector despite antibody production.

## 1.2 INGN 201

INGN 201 is a replication defective adenoviral vector that encodes a wild-type p53 gene driven by the CMV promoter. The INGN 201 backbone is an $E_1$-partial $E_3$- deleted human adenovirus Type 5 serotype. $E_1$ and $E_3$ gene products modulate viral replication and host immune response.

Two Phase I/II studies of intratumoral INGN 201 have been completed. A total of 604 doses have been given to 86 patients. While the maximum tolerated dose was not reached in either study, repeated intratumoral injections of up to $3x10^{12}$ viral particles (vp) have been well tolerated. The most common adverse events thought to be related to INGN 201 were pain at the site of injection (25 patients), fever (24 patients) and nausea (7 patients). All events were mild to moderate in intensity and had resolved by the end of the studies. In addition to being safe, INGN 201 has demonstrated objective activity in lung and head and neck cancers.